NMR SPECTROSCOPY METHOD

This invention is concerned with nuclear magnetic resonance (NMR) spectroscopy and/or NMR imaging. The technique involves observing the spectrum/image of a NMR active nuclear species *in vitro* in order to obtain information about the environment in which the species is present. The spectra of NMR active nuclei vary depending on their environment, and this is reported in the literature (PNAS, 93,12932-6, 1996).

Noble gases having non-zero nuclear spin can be hyperpolarised, i.e. have their polarisation enhanced over the equilibrium polarisation, e.g. by the use of circularly polarised light. Preferred techniques for hyperpolarisation include spin exchange with an optically pumped alkali metal vapour and metastability exchange. Noble gases to which this technique can be applied include ^{3}He and ^{129}Xe. As described by M S Albert *et al* in US Patent 5,545,396, the technique can be used to prepare hyperpolarised noble gases that can be administered by inhalation for magnetic resonance imaging of the human body.

Xenon is chemically inert and has hydrophobic properties, and is capable of being weakly bound by hydrophobic regions of biological molecules (PNAS, 78, No 8, 4946-9, August 1981; Abstracts of the 11th Annual Meeting of the Society for Magnetic Resonance in Medicine (1992) page 2104). Thus it is possible to "label" biological molecules with xenon.

This invention concerns the method of labelling biological molecules with hyperpolarised ^{129}Xe. All macromolecules have a number of discrete hydrophobic and hydrophilic sites. Xenon will bind by hydrophobic interactions to hydrophobic sites with different affinity. The xenon labels the biological compound by becoming weakly bound to it, e.g. at specific hydrophobic sites on a surface of or within a cavity of a protein or other macromolecule.

The NMR sensitivity of hyperpolarised xenon is highly increased compared to non-hyperpolarised xenon. Another advantage of the present invention is the reversible and non-destructive nature of the bond between the xenon and the biological molecule. A further advantage is that the forming of the "bond" and subsequent measurement may be repeated if

needed. In addition, since xenon is a gas (condensation temperature of -106°C), it and may easily and rapidly be separated from the biological molecule if necessary. Moreover, xenon is essential chemically inert and will not adversely effect the biological molecule.

One embodiment of the invention thus provides an *in vitro* method which comprises labelling a biological molecule with hyperpolarised xenon, and observing a magnetic resonance spectrum and/or image of the hyperpolarised xenon in the environment of the biological molecule. The spectrum/image provides information about the environment(s) at which atoms of xenon are bound to the biological molecule. Any conformational change of the biological molecule resulting e.g. from the binding (or the disappearance) of a ligand (e.g. a lipid, carbohydrate, peptide, polypeptide, nucleic acid or any sort of drug) or cleavage by an enzyme, will cause an alteration in the xenon NMR spectrum. Each hydrophobic site in the biological molecule may give rise to a specific and characteristic NMR shift.

A further embodiment of the present invention is to take NMR "fingerprint(s)" of a known biological molecule. These fingerprints can subsequently be used to identify unknowns by direct comparison in a manner similar to infra-red spectroscopy.

A biological molecule as defined by the present invention is a monomeric or polymeric molecule that is present in biological systems or that is artificially introduced and is biologically active in such systems. Biological molecules include lipids, sugars and polysaccharides, nucleic acids (DNA, RNA), nucleosides, oligonucleosides, polynucleosides, nucleotides, oligonucleotides, polynucleotides, enzymes, vitamins and particularly peptides, polypeptides and proteins.

In one preferred embodiment of the invention, the labelled biological molecule is an assay reagent taking part in an assay method and wherein the assay reagent is labelled with hyperpolarised xenon. The labelling of the biological molecule with hyperpolarised xenon may be performed before, during or after performance of the assay.

An assay method according to the present invention is a test involving a reaction of one or more biological molecules. The assays include for example competition assays (e.g. receptor-

ligand antagonism, enzyme-substrate inhibitors, protein-protein interaction inhibitors), binding assays (e.g. receptor-ligand agonism, enzyme-substrate reactions, protein-protein interactions), immunoassays (e.g. for specific analytes), hybridisation assays (e.g. nuclease assays, mutation analysis, mRNA and DNA detection), test involving cells, organs and/or whole organisms. These tests may involve e.g. one or more lipids, saccharides, polynucleotides, oligonucleotides, nucleotides, peptides or proteins. Assays include binding studies performed on eukaryotic and prokaryotic microorganisms; binding studies performed on tissue *in vitro*; and binding studies in which an assay reagent is administered *in vivo* and an excretion product (e.g. urine, faeces, or breath) analysed by NMR *in vitro*.

By observing a change with time using NMR, the progress of a reaction can be followed during the course of an assay. Assays performed *in vitro* may conveniently be in multiwell plates, with either an assay reagent in the wells of the plate being labelled with hyperpolarised xenon, or a reagent being so labelled in bulk prior to being dispensed into individual wells of the plate.

Generally the biological molecule is present in a liquid medium into which the xenon is introduced as a gas. This may be achieved e.g. by bubbling it through the fluid or by contact with the biological molecule as a solid. Alternatively the xenon is introduced as a solution in a solvent, which is compatible with the biological molecule (e.g. in a lipophilic solvent such as a lipid or a fluorocarbon solvent).

The liquid medium used according to one embodiment of the present invention may be deuterated water, deuterated buffers or solvents, e.g. lipophilic solvents which may contain lipid bicelles, lipid vesicles, liposomes, cryptophanes and/or cyclodextrins.

^{129}Xe has a natural abundance of 26.4%. The xenon used for this invention may be either the naturally occurring material or one artificially enriched in ^{129}Xe. A preferred degree of enrichment ^{129}Xe is 40 % or more. A more preferred degree is 50 % or more and an even more preferred degree is 75 % or more. A particularly preferred degree of enrichment is 90 % or more. Bulk supplies of xenon enriched in ^{129}Xe and hyperpolarised to a high degree are now available commercially and have a half life long enough to permit transport over substantial

distances. While the half life of hyperpolarised ^{129}Xe in the biological environments contemplated in this invention will be lower, it is expected to be amply sufficient to permit the desired spectra to be obtained. A preferred degree of hyperpolarisation is 8 % or more. A more preferred hyperpolarisation degree is 20 % or more and an even more preferred degree is 30 % or more. Ideally, the degree will approach 100 %.

In one embodiment of the invention, the temperature at the time xenon is added is above the temperature at which the biological molecule is frozen, but below the temperature at which the biological molecule may be denatured. Alternatively, xenon may be added to the frozen biological molecule, followed by thawing. However, the right temperature to achieve the optimal function of the biological molecule should also be considered.

In one embodiment of the invention, the solution is kept as low as possible in order to slow down the exchange between the bound xenon and free xenon, without broadening the NMR signals too much.

In a further embodiment of the invention, the solution is made viscous due to the use of one viscous solvent or the use of a suitable combination of solvents. The viscosity of the solvent is preferably within the range of 500 mPs to 5000 mPs, more preferably within the range of 700 mPs to 1500 mPs.

In one embodiment of the invention, the pressure of xenon is as high as possible, preferably higher than 5 $x10^5$ N/m^2 (5 bar), more preferably higher than 5 x 10^6 N/m^2 (50 bar), even more preferably higher than 1 $x10^7$ N/m^2 (100 bar) and particularly higher than $2x10^7$ N/m^2 (200 bar). However, the pressure must never be so high that the biological molecule will be adversely effected.

The invention is illustrated with reference to the following non-limiting Example.

Hyperpolarised ^{129}Xe is generated by optical pumping as described by B.Driehuys et al., Appl.Phys.Lett. 69 (12), 1996. The Isotopic composition of the gas is 80% ^{129}Xe and 0.25% ^{131}Xe (the rest non-magnetic isotopes of Xe). The degree of polarisation is estimated to be 10%.

Lyzozyme (28 mg) is dissolved in a mixture of D_2O and methanol-d_4 (40:60) (3 ml) in a heavy-walled 10 mm NMR-tube. This mixture is subjected to four freeze-pump-thaw cycles of degassing. The tube is then connected to the outlet of the polariser and frozen in liquid nitrogen. The hyperpolarized gas is generated and collected on a cold finger at liquid nitrogen temperature in a holding field of 200 mT over a period of 15 minutes which is estimated to give a volume of 50 ml of Xe at NTP. A narrow Dewar vessel with liquid nitrogen is placed in a magnet with a field strength of 0.3 T. The collected xenon is thawed and then refrozen in the NMR-tube in the 0.3 T magnet. The sample tube is flame-sealed and the frozen sample is moved to the fringe field of the magnet of an NMR-spectrometer. The NMR-spectrometer sample space is kept at a temperature of 293 K. The sample is removed from the transport magnet and thawed by heating with the hand (protected from the cold) while standing as close to the NMR-magnet as possible. When the sample starts to thaw it is shaken vigorously and inserted into the spectrometer. A ^{129}Xe spectrum is recorded and apart from the large peak due to the bulk xenon, a small peak, with a line width of 160 Hz, due to bound xenon can be observed at -158 ppm relative to bulk xenon.